SUPPORTING INFORMATION

Reduction of Aromatic and Heterocyclic Aromatic *N*-Hydroxylamines by Human Cytochrome P450 2S1

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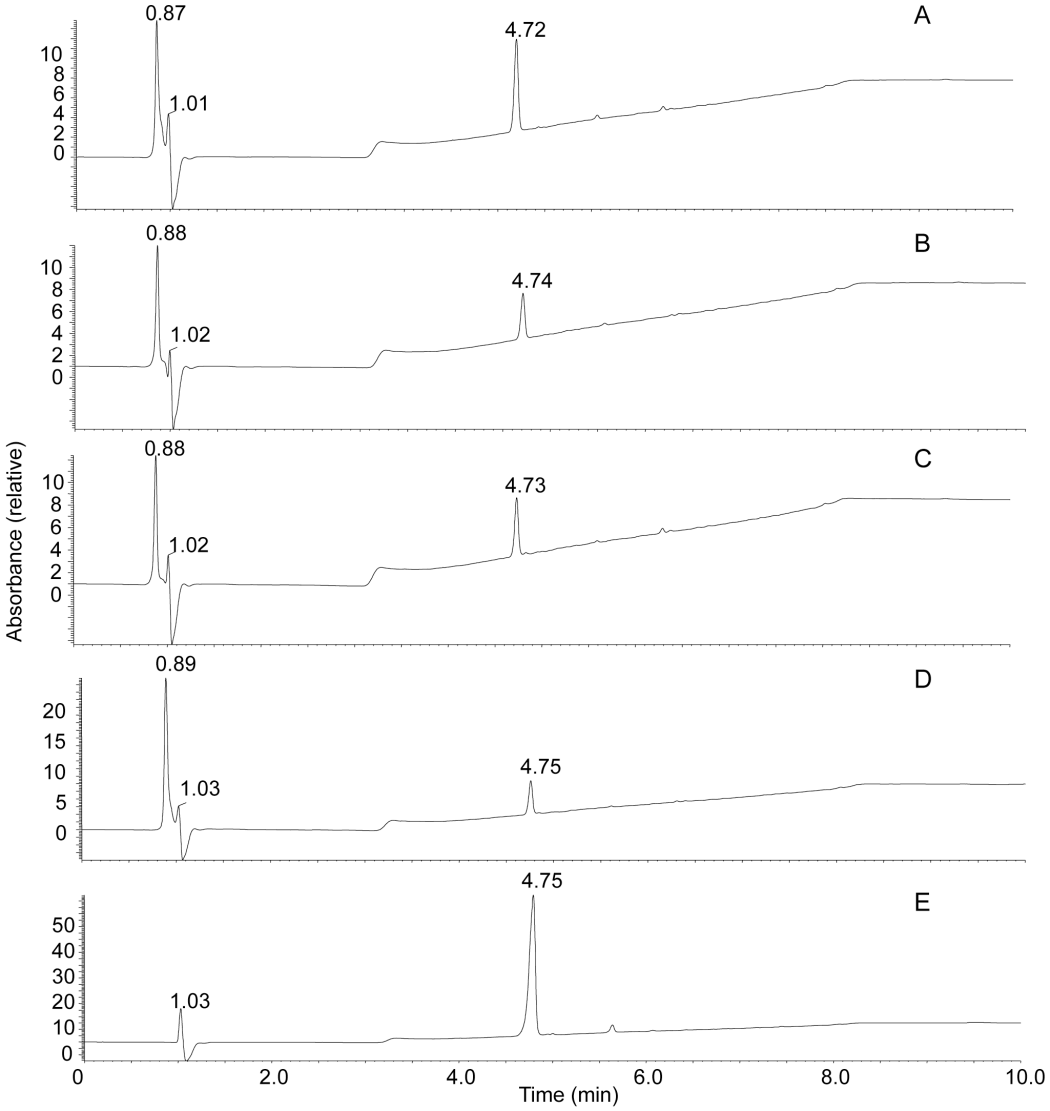
Figure S11. UV spectra of 2-AF, 2-HONH-fluorene, 2-NO-fluorene, 2-NO_2-fluorene, and azoxyfluorene.

Figure S12. HPLC chromatograms of anaerobic incubations of HONH-5F 203 with P450 1A1 and 2W1.

Figure S13. HPLC chromatograms of anaerobic incubations of 4-HONH-biphenyl with P450 1A1, 1A2, 2S1, 2W1, and 3A4.

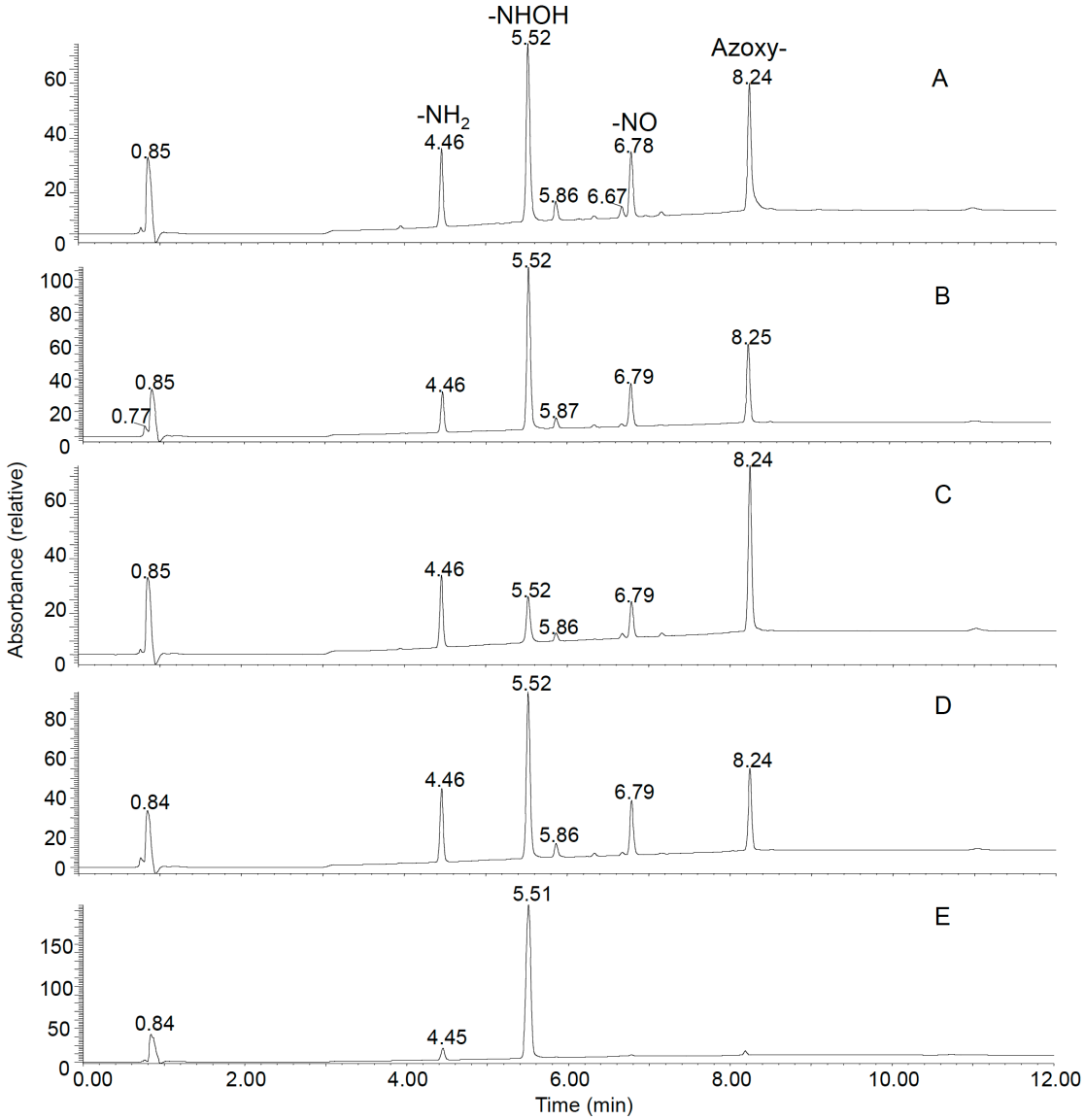
Figure S14. HPLC chromatograms of anaerobic incubations of 2-HONH-fluorene with P450 1A1, 1A2, 2S1, 2W1, and 3A4.

Figure S1. Anaerobic incubations of HONH-phenacetin with P450 2S1.



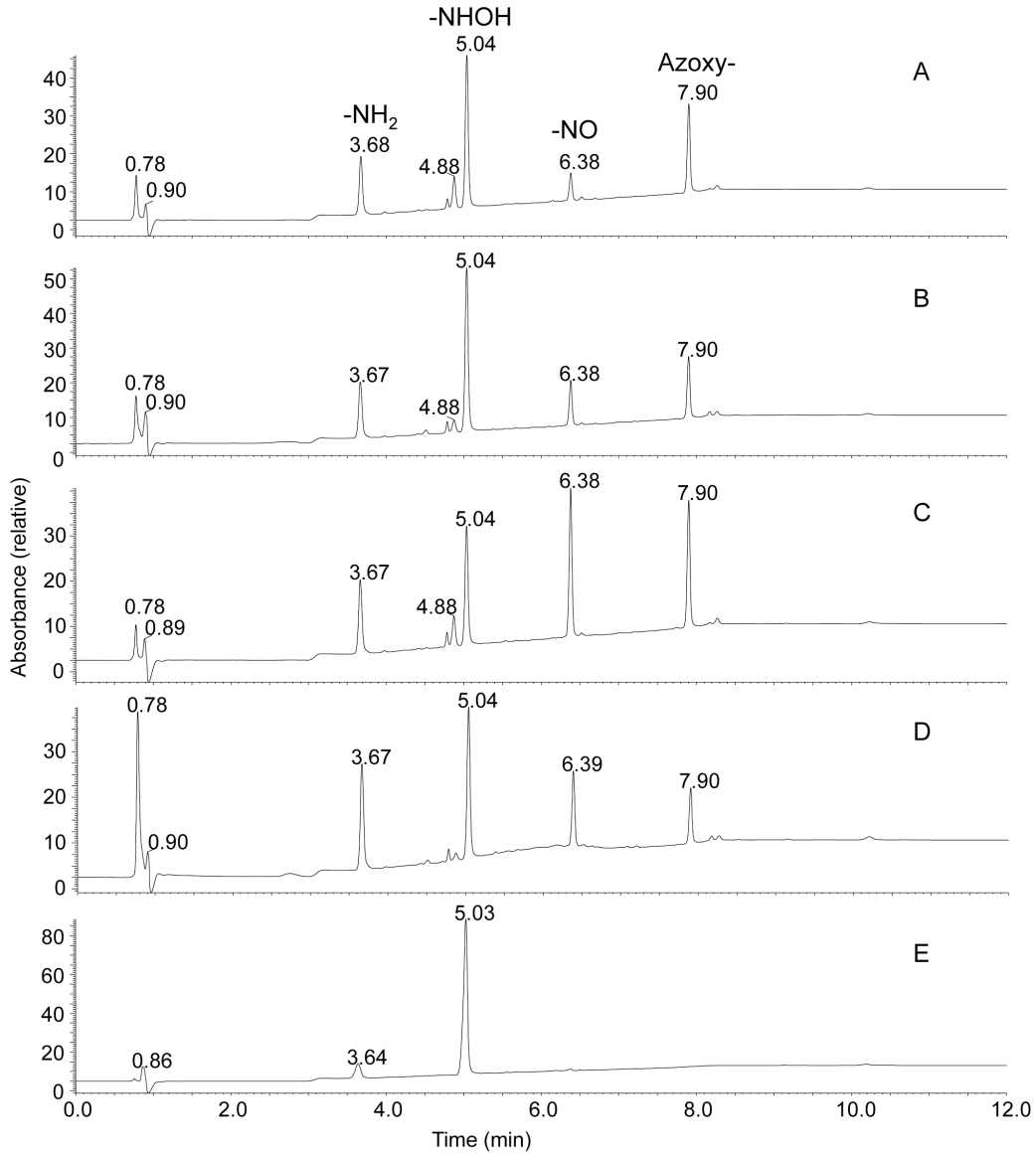
Under anaerobic conditions, HONH-phenacetin (t_R 4.7 min) was (A) incubated in 100 mM sodium HEPES buffer (pH 7.4) containing 1 mM EDTA at 37 °C for 20 min; (B) incubated with all P450 system components with the exception of P450 2S1; (C) incubated with all components of the P450 2S1 system, with the exception of the NADPH-generating system; (D) incubated with P450 2S1 in the presence of NPR and an NADPH-generating system. (E) Synthetic 4-HONH-biphenyl standard. UV absorbance was integrated over the range 200-400 nm.

Figure S2. HPLC chromatograms of aerobic incubations of 4-HONH-biphenyl with P450 2S1.



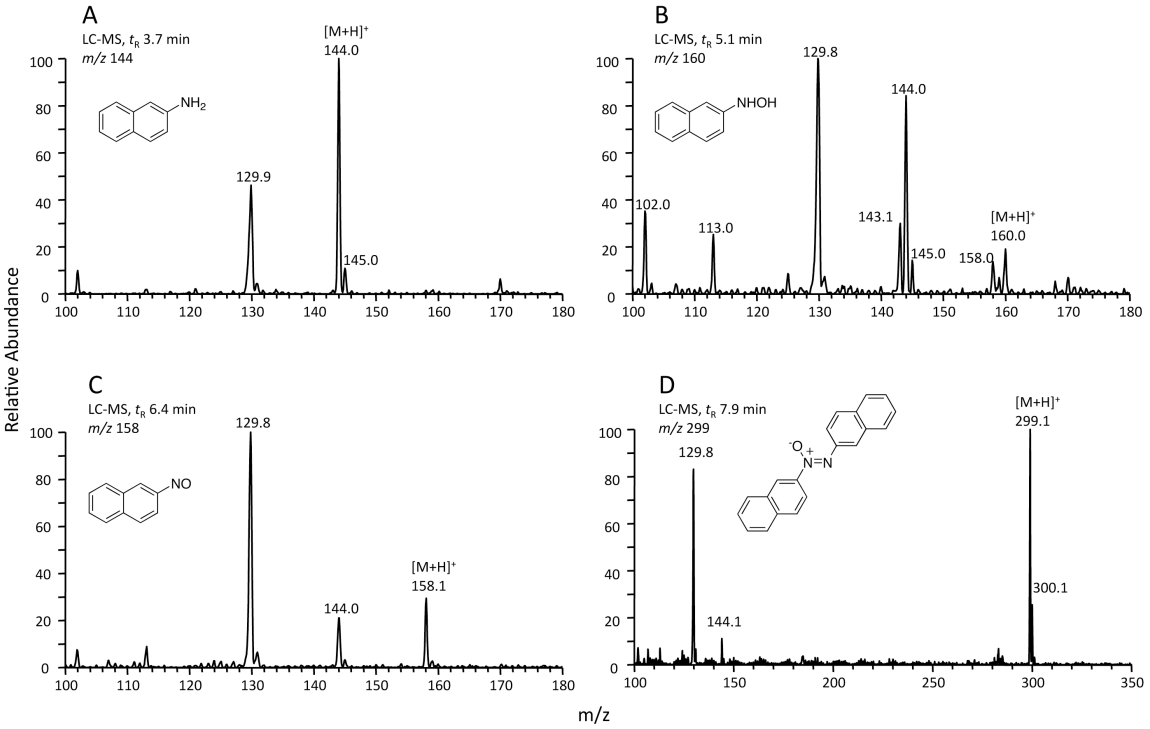
Aerobic incubations of 4-HONH-biphenyl with P450 2S1 under conditions the same as in anaerobic incubations. The contribution to the formation of 4-ABP by P450 2S1 is not apparent. Similar amounts of 4-ABP were produced under different incubation conditions. However, in the presence of P450 2S1, NPR, and an NADPH-generating system, the least amount of azoxybiphenyl was formed, indicating that the least amount of 4-ABP was formed due to the disproportionation of 4-HONH-biphenyl; i.e. a portion of formation of 4-ABP might be attributed to the reduction of 4-HONH-biphenyl by P450 2S1. (A) 4-HONH-biphenyl in sodium HEPES buffer. (B) Incubation without P450 2S1. (C) Incubation without the NADPH-generating system. (D) Incubation with P450 2S1, NPR, and an NADPH-generating system. (E) Synthetic standard of 4-HONH-biphenyl. UV absorbance was integrated over the range 200-400 nm.

Figure S3. HPLC chromatograms of anaerobic incubations of 2-HONH-naphthalene with P450 2S1.



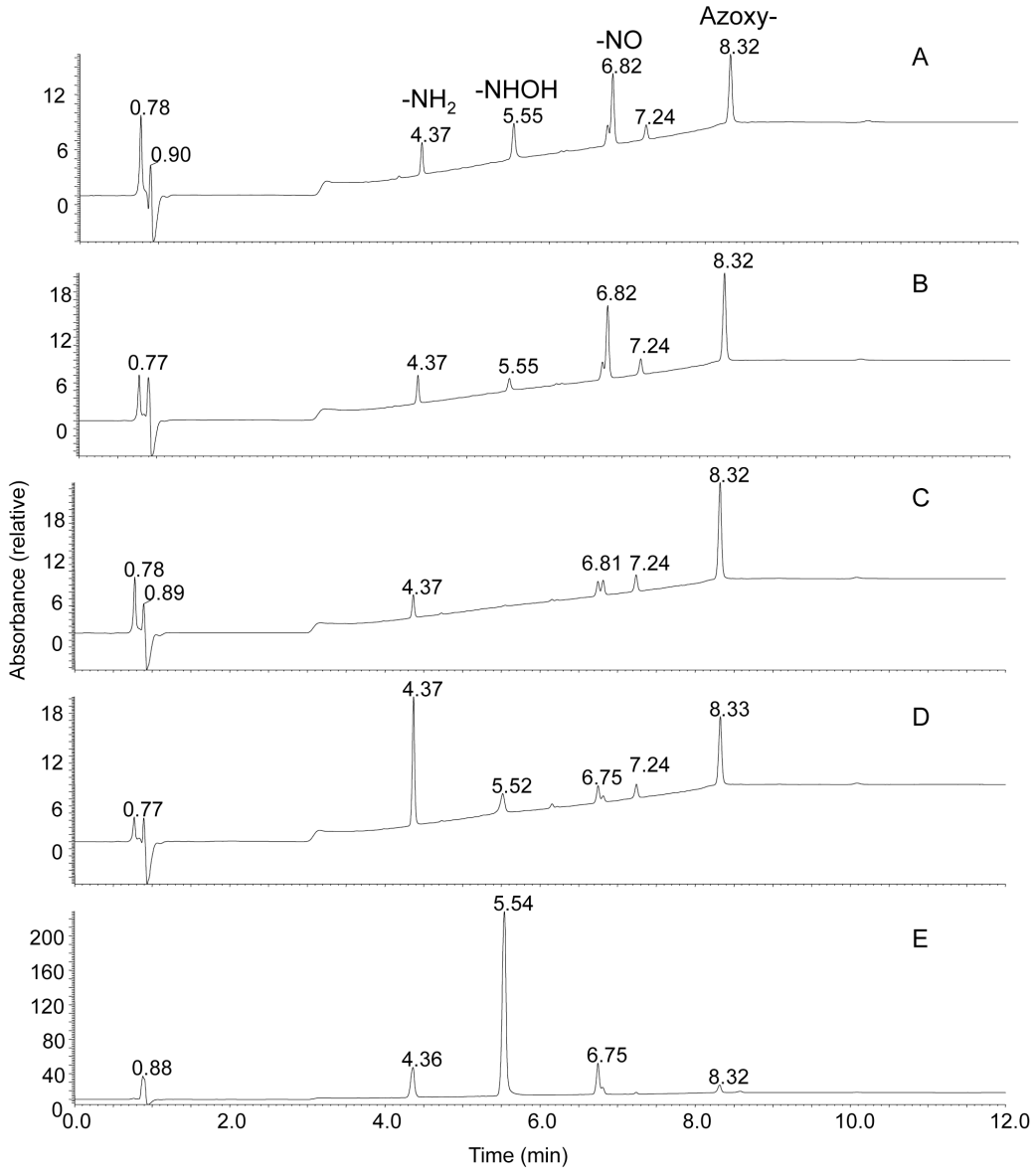
Freshly prepared 2-HONH-naphthalene (5 μL of a 10 mM solution in $(CH_3)_2SO$) was incubated with P450 2S1 (total volume 0.5 mL) at 37 °C for 10 min under anaerobic conditions. (A) 2-HONH-naphthalene in 100 mM sodium HEPES buffer (pH 7.4) containing 1 mM EDTA, incubated at 37 °C for 10 min. (B) 2-HONH-naphthalene incubated with all P450 system components with the exception of P450 2S1. (C) 2-HONH-naphthalene incubated with all components of the P450 2S1 system with the exception of the NADPH-generating system. (D) 2-HONH-naphthalene incubated with P450 2S1 in the presence of NPR and an NADPH-generating system. (E) Synthetic standard of 2-HONH-naphthalene. UV absorbance was integrated over the range 200-400 nm.

Figure S4. Mass spectra of incubation products of 2-HONH-naphthalene with P450 2S1.



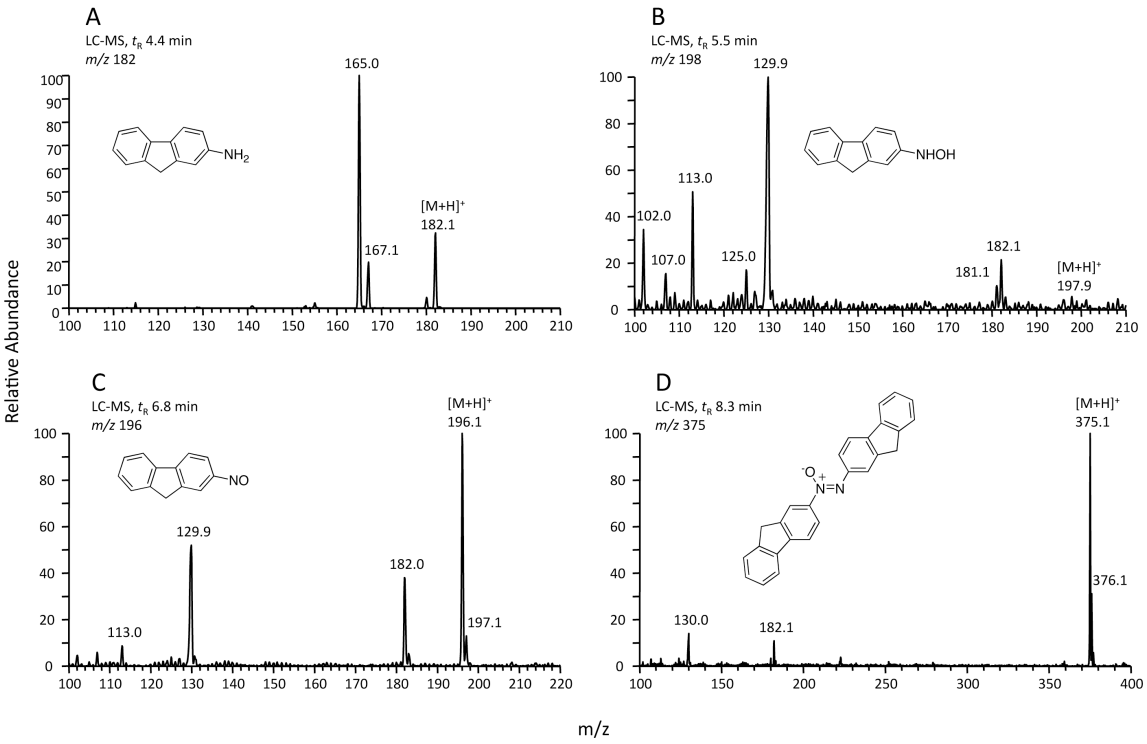
(A) 2-NA (t_R 3.1 min). (B) 2-HONH-naphthalene (t_R 5.7 min). (C) 2-NO-naphthalene (t_R 6.4 min). (D) Azoxynaphthalene (7.9 min).

Figure S5. HPLC chromatograms of anaerobic incubations of 2-HONH-fluorene with P450 2S1.



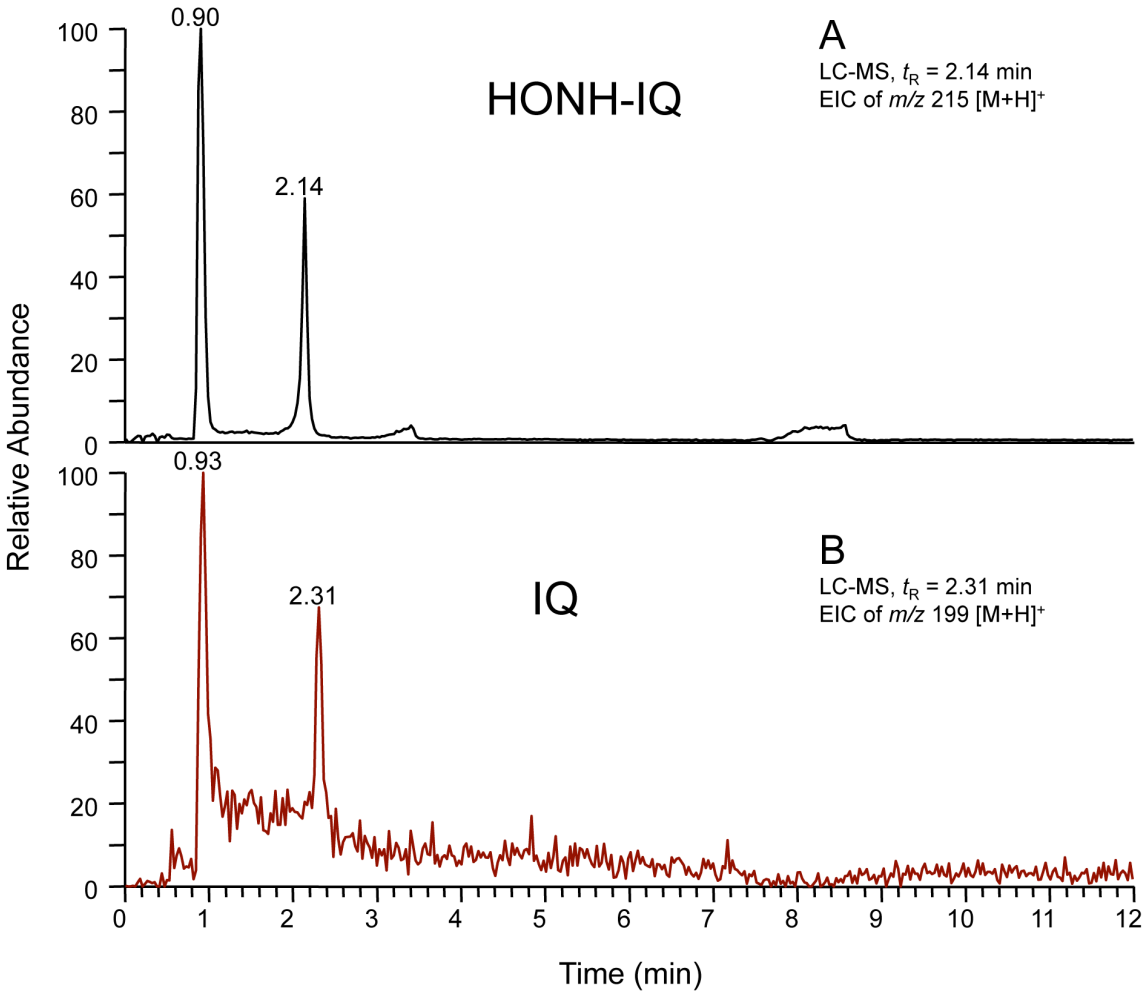
Freshly prepared 2-HONH-fluorene (5 μL of a 10 mM solution in $(CH_3)_2SO$) was incubated with P450 2S1 (total volume 0.5 mL) at 37 °C for 15 min under anaerobic conditions. (A) 2-HONH-fluorene in 100 mM sodium HEPES buffer (pH 7.4) containing 1 mM EDTA, incubated at 37 °C for 15 min. (B) 2-HONH-fluorene incubated with all P450 system components with the exception of P450 2S1. (C) 2-HONH-fluorene incubated with all components of the P450 2S1 system with the exception of the NADPH-generating system. (D) 2-HONH-fluorene incubated with P450 2S1 in the presence of NPR and an NADPH-generating system. (E) Synthetic standard of 2-HONH-fluorene. UV absorbance was integrated over the range 200-400 nm.

Figure S6. Mass spectra of incubation products of 2-HONH-fluorene with P450 2S1.



(A) 2-AF (t_R 4.4 min). (B) 2-HONH-fluorene (t_R 5.5 min). (C) 2-NO-fluorene (t_R 6.8 min). (D) Azoxyfluorene (8.3 min).

Figure S7. LC-MS chromatograms of HONH-IQ and IQ.



(A) Extracted ion chromatogram of *m/z* 215 ($[M+H]^+$, from incubation of NO_2-IQ with P450 2S1, HONH-IQ, t_R 2.14 min). (B) Extracted ion chromatogram of *m/z* 199 ($[M+H]^+$, from incubation of NO_2-IQ with P450 2S1, IQ, t_R 2.31min).

Figure S8. UV spectra of 5F 203, HONH-5F 203, and NO-5F 203

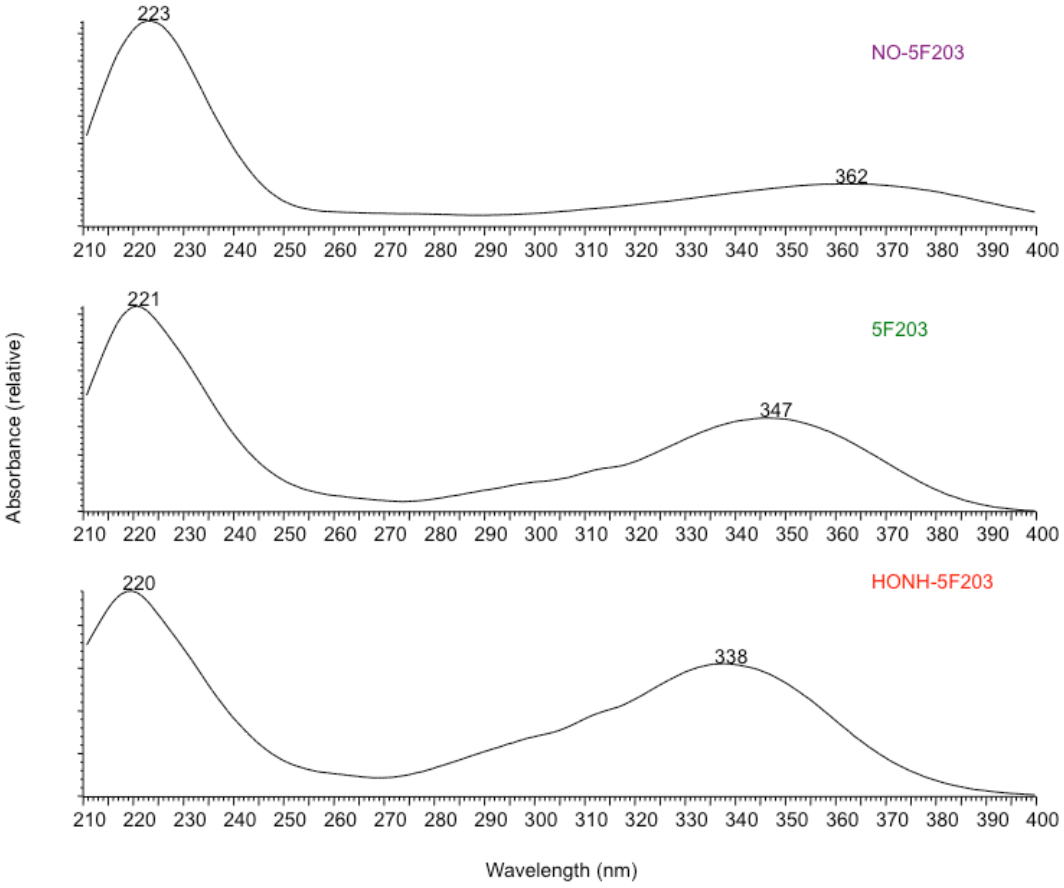


Figure S9. UV spectra of 4-ABP, 4-HONH-biphenyl, 4-NO-biphenyl, 4-NO_2-biphenyl, and azoxybiphenyl.

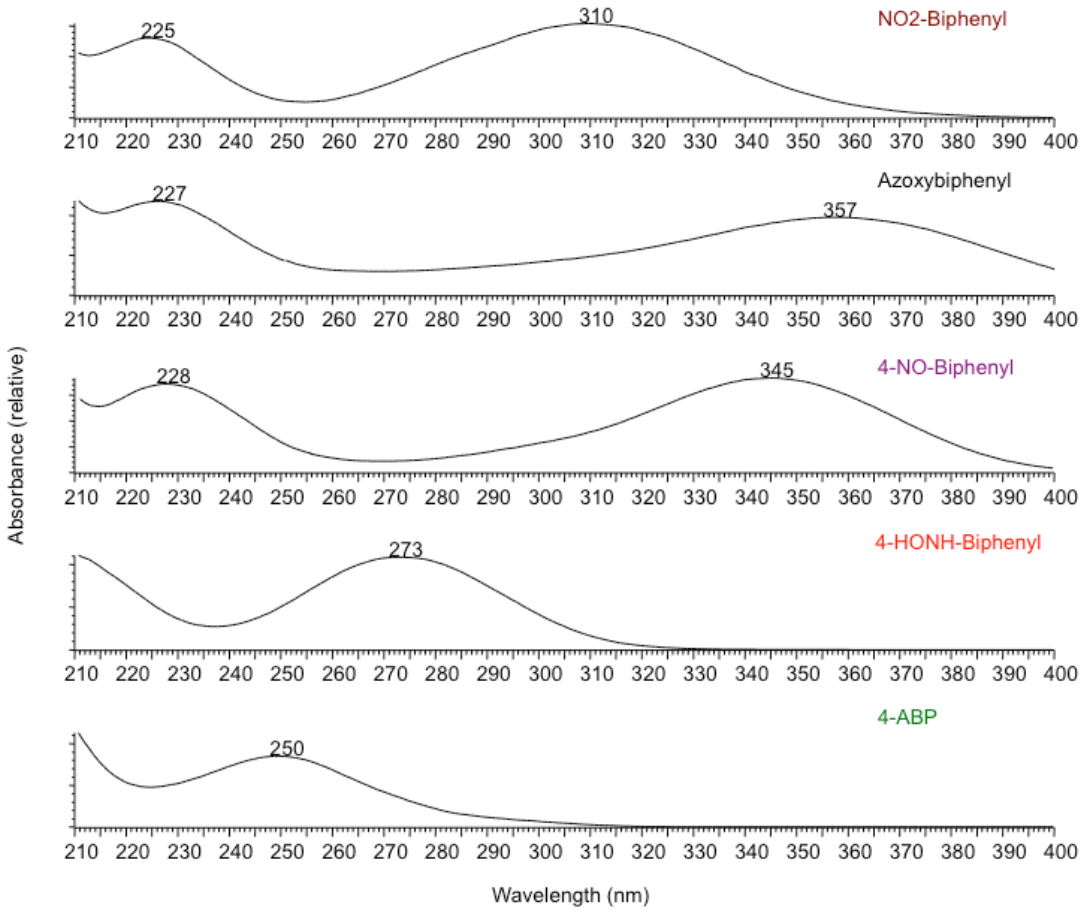


Figure S10. UV spectra of 2-NA, 2-HONH-naphthalene, 2-NO-naphthalene, 2-NO_2-naphthalene, and azoxynaphthalene.

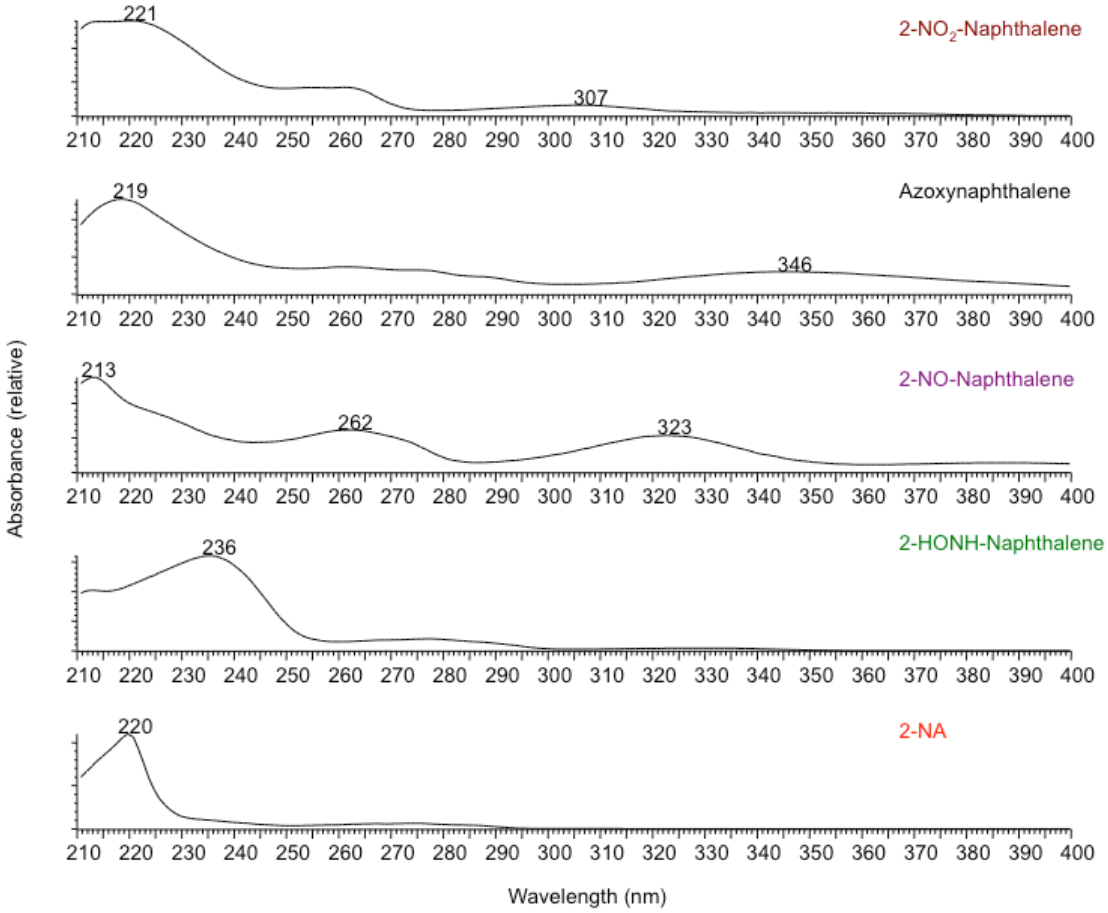


Figure S11. UV spectra of 2-AF, 2-HONH-fluorene, 2-NO-fluorene, 2-NO_2-fluorene, and azoxyfluorene.

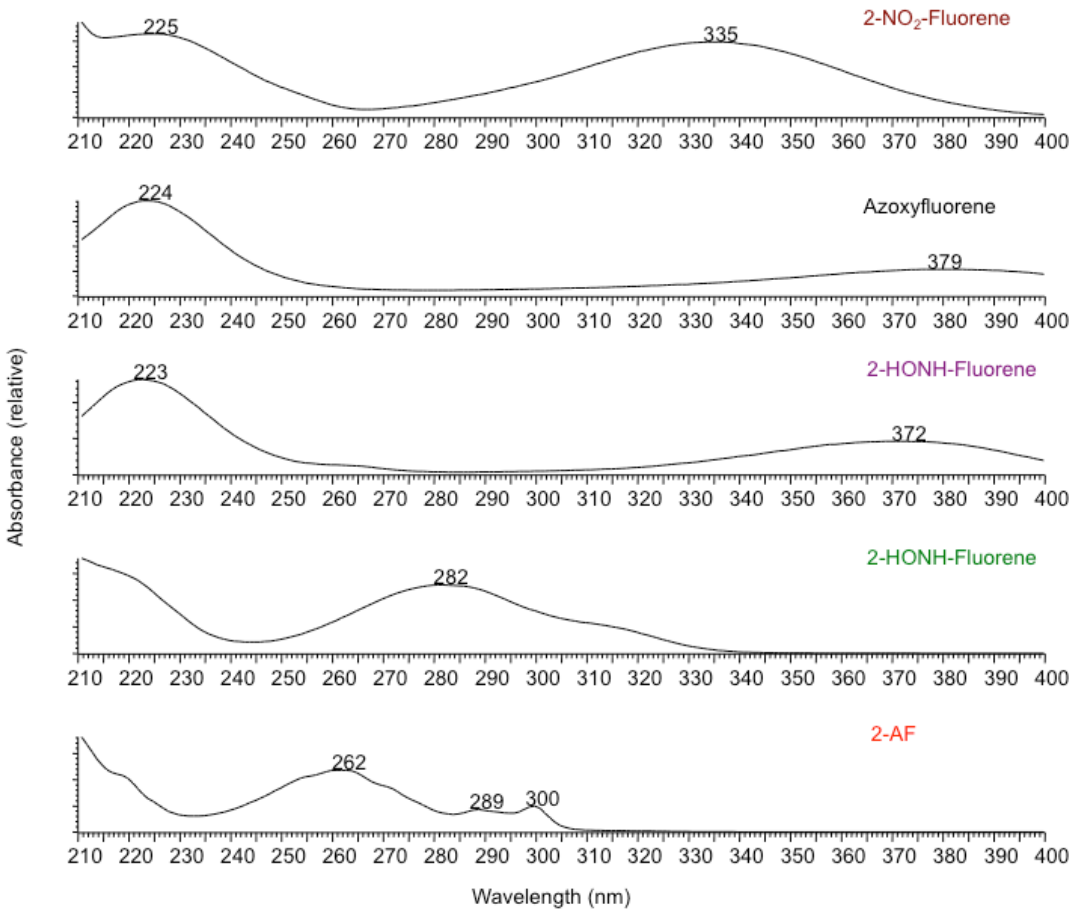
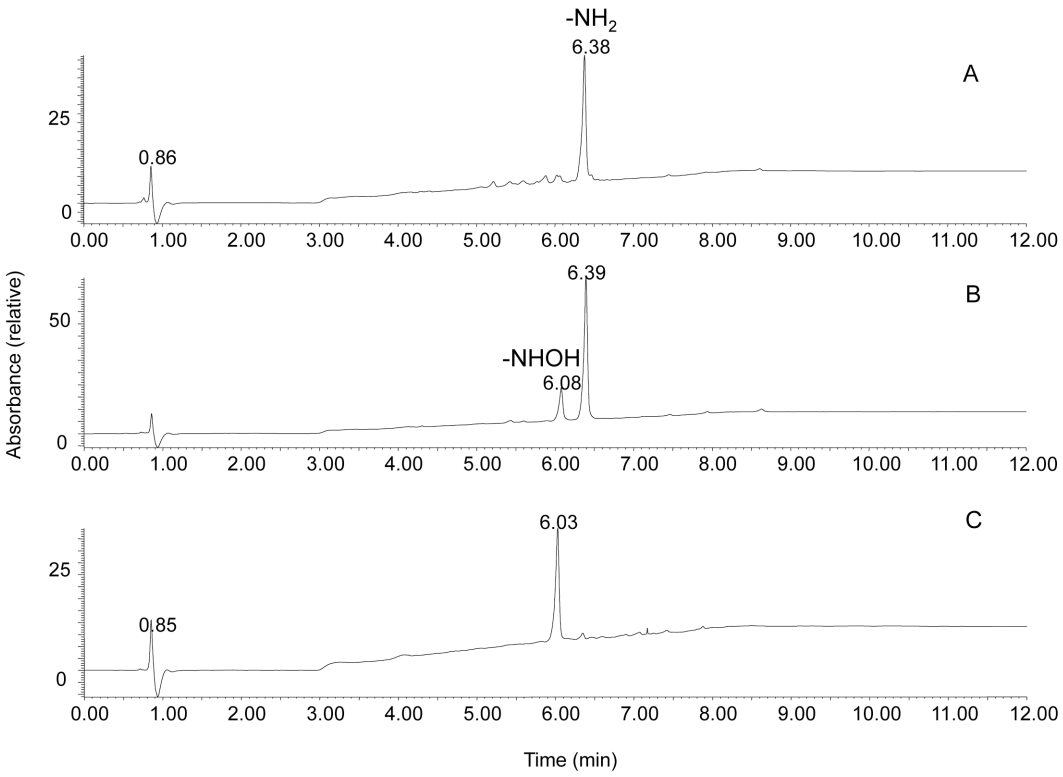
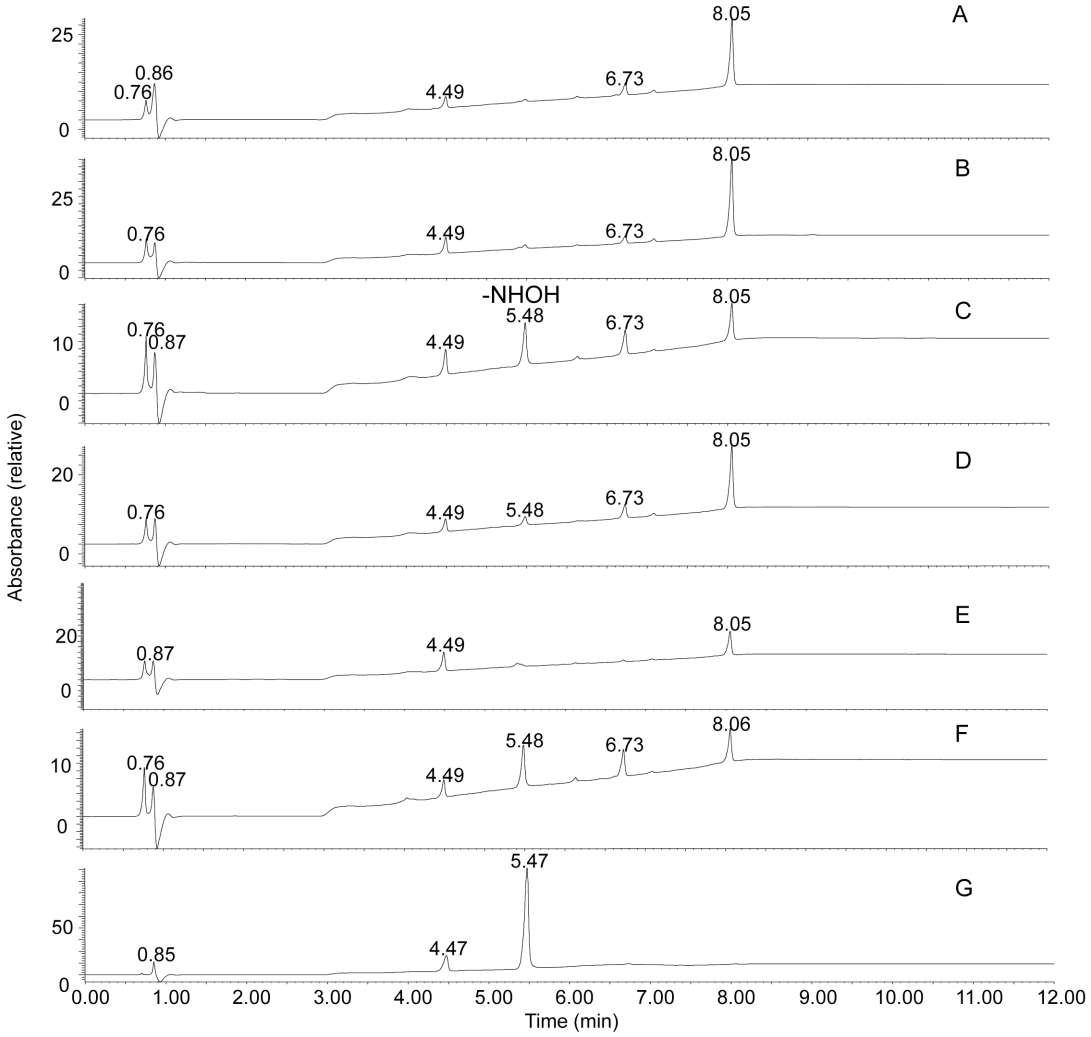


Figure S12. Anaerobic incubations of HONH-5F 203 with P450 1A1 and 2W1.



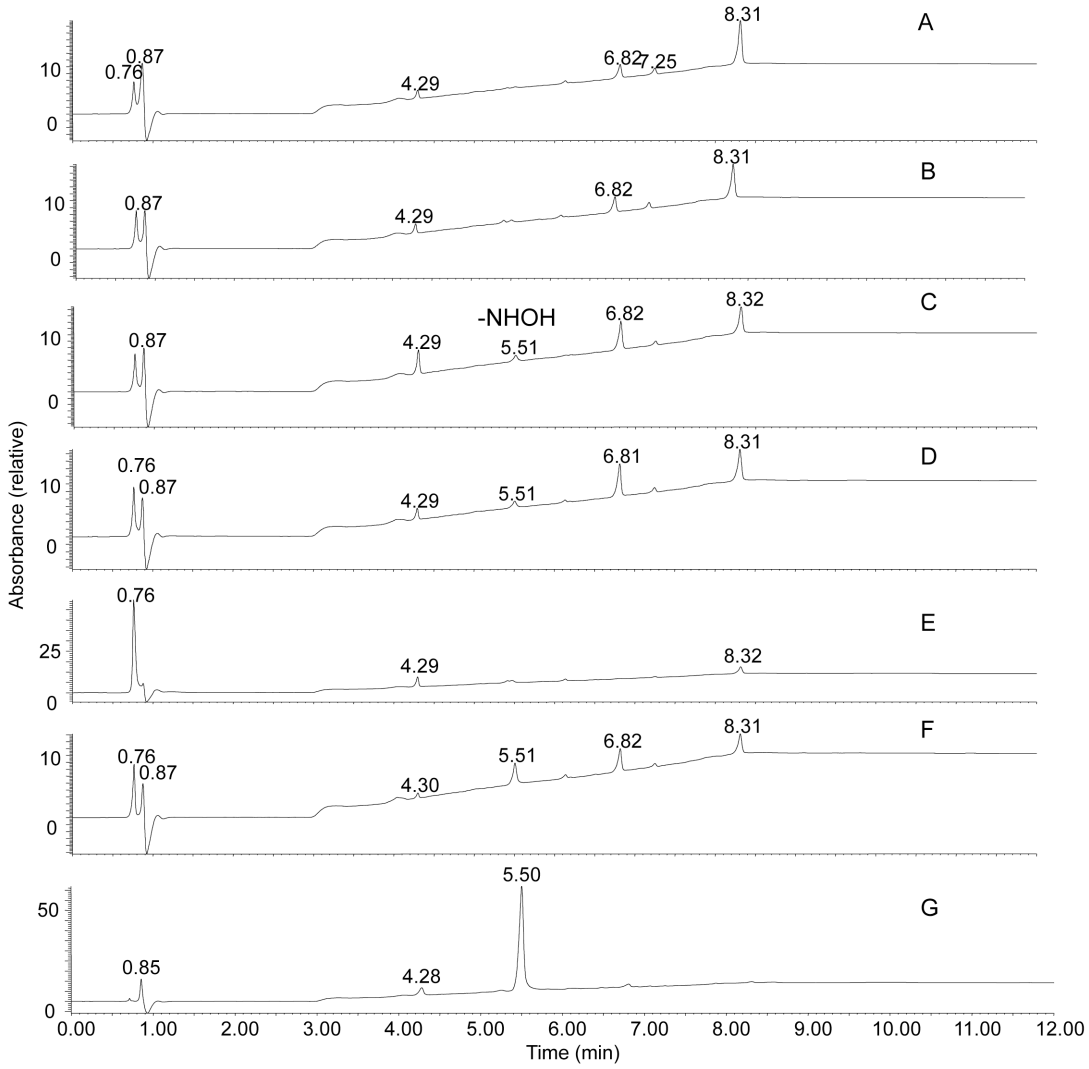
Under anaerobic conditions, HONH-5F 203 (t_R ~6.1 min) was incubated in 100 mM sodium HEPES buffer (pH 7.4) containing 1 mM EDTA at 37 °C for 30 min, in the presence of NPR and an NADPH-generating system (A) with P450 1A1 or (B) with P450 2W1. (C) Synthetic standard of HONH-5F 203. UV absorbance was integrated over the range 200-400 nm.

Figure S13. Anaerobic incubations of 4-HONH-biphenyl with P450 1A1, 1A2, 2S1, 2W1, and 3A4.



Under anaerobic conditions, HONH-biphenyl (t_R 5.5 min) was incubated in 100 mM sodium HEPES buffer (pH 7.4) containing 1 mM EDTA at 37 °C for 10 min, in the presence of NPR and an NADPH-generating system with P450 (A) 3A4; (B) 2W1; (C) 2S1; (D) 1A2; (E) 1A1. (F) Anaerobic incubation of 4-HONH-biphenyl in sodium HEPES buffer. (G) Synthetic standard of 4-HONH-biphenyl. UV absorbance was integrated over the range 200-400 nm.

Figure S14. Anaerobic incubations of 2-HONH-fluorene with P450 1A1, 1A2, 2S1, 2W1, and 3A4.



Under anaerobic conditions, 2-HONH-fluorene (t_R 5.5 min) was incubated in 100 mM sodium HEPES buffer (pH 7.4) containing 1 mM EDTA at 37 °C for 10 min, in the presence of NPR and an NADPH-generating system with P450 (A) 3A4; (B) 2W1; (C) 2S1; (D) 1A2; (E) 1A1. (F) Anaerobic incubation of 2-HONH-fluorene in sodium HEPES buffer. (G) Synthetic standard of 2-HONH-fluorene. UV absorbance was integrated over the range 200-400 nm.